

Southwest Fisheries Science Center
Administrative Report H-92-07C

**OCCURRENCE OF POTENTIAL PATHOGENS IN GREEN SEA TURTLES
(*Chelonia mydas*) AFFLICTED OR FREE OF FIBROPAPILLOMAS
IN KANEOHE BAY, ISLAND OF OAHU, HAWAII, 1991.**

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NOT FOR PUBLICATION

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PREFACE

Prepared under contract as part of the Southwest Fisheries Science Center Honolulu Laboratory's research program on threatened and endangered species of marine turtles, this report provides an increased understanding of potentially significant pathological bacteria and other selected disease agents in the green turtle, *Chelonia mydas*, associated with the formation of tumors known as fibropapillomas. The incidence of these life-threatening tumors on green turtles in the Hawaiian Islands has increased to epidemic proportions during recent years. A similar situation exists among green turtles at certain sites in Florida, the Caribbean, and other selected locations worldwide. The cause of fibropapillomas in green turtles remains unknown. The impact of the disease on the afflicted populations can have serious consequences and represents one more threat to the survival of the green turtle. The nature of this disease and its cause must be determined in order to develop a long-term disease management program. The present report by Dr. Alonso Aguirre constitutes progress in that direction which must be followed up with additional studies.

Because this report was prepared by an independent investigator, its statements, findings, conclusions and recommendations do not necessarily reflect the views of the National Marine Fisheries Service, NOAA.

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June 1992

EXECUTIVE SUMMARY

Thirty-two juvenile green sea turtles (*Chelonia mydas*) were trapped in Kaneohe Bay, Island of Oahu, Hawaii, during September 1991. Thirty-one percent of the turtles sampled were afflicted with green turtle fibropapillomas (GTFP) in varying degrees of severity. Virus isolation attempts were negative in all individuals. A beginning data base on the bacterial flora of green turtles afflicted and free of GTFP is provided. Nasopharyngeal and cloacal swabs taken yielded 28 gram negative bacteria, 5 gram positive cocci, *Bacillus* spp., and diphtheroids. The most common isolates included *Pseudomonas fluorescens* (68%), *P. putrefaciens* (66%), *Vibrio alginolyticus* (50%), non-hemolytic *Streptococcus* (50%), *V. damsela* (47%), and *V. fluvialis* (47%). Chlamydial antigen was detected in 12.5% of the turtles sampled. No hemoparasites were observed in thin blood smears analyzed. In addition, light and electron microscopy were performed in tumor biopsies, providing histopathologic characterization of GTFP and other parasitologic findings. Although the etiologic agent of GTFP was neither isolated nor characterized, this pilot study provided new information and possible insights in identifying the etiology of GTFP. Further research related to basic parasitologic, pathologic, toxicologic, and epidemiologic studies is recommended.

INTRODUCTION

Green turtle fibropapillomatosis (GTFP) is a condition affecting the green sea turtle (*Chelonia mydas*) in several parts of the world, including Australia, the Bahamas, Barbados, Belize, Cayman Islands, Dominican Republic, Culebra Island, Curacao, Colombia, Florida, Hawaii, Mexico, Panama, Puerto Rico, St. Croix, St. Thomas, Venezuela, and the Virgin Islands (Jacobson et al. 1991, Williams et al. 1991). The primary concern of the GTFP Research Plan (Balazs and Pooley 1991) is to study the etiology and epidemiology of this disease, specifically to determine the effects of environmental, genetic, biological, and behavioral factors affecting green turtles. The ultimate goal is to find a solution to contain GTFP in sea turtle populations.

Although a number of recent promising research findings have suggested a papovavirus as the causative agent, the primary etiology remains to be elucidated. Other possible etiological agents have been suggested, including an immune response to trematode ova, secretion of hirudin by marine leeches, other viruses such as a herpesvirus, excessive solar radiation, chemical pollutants impairing the immune system, stress, and a genetic predisposition to neoplasia.

Evidence of infection to other potential pathogens epidemiologically linked to the etiologic agent of GTFP is necessary to provide information on the health status of a population in endemic areas affected by GTFP. Surveillance of currently known diseases and application of other isolation techniques to identify new etiologic agents should be considered (Aguirre 1991).

The purpose of this pilot study was the development of a disease survey to identify potential pathogenic bacteria and other disease agents possibly associated with GTFP in a discrete population of green sea turtles afflicted or free of GTFP on Oahu.

MATERIALS AND METHODS

Study Area

Oahu's Kanehoe Bay (long. 21°30'N, lat. 157°50'W), Island of Oahu, Hawaii, is the largest bay in the Hawaiian islands comprising substantial foraging and resting habitat for green turtles. The earliest confirmed case of GTFP in Hawaii was reported in Kanehoe Bay in 1958. In this bay, live capture by hand has shown GTFP rates of from 49 to 92% since February 1989 (Balazs 1991).

Field Techniques

A total of 32 green turtles were captured live by hand using scuba (Balazs 1985) in Kanehoe Bay during 20-26 September 1991. Turtles were trapped in three areas: Ahu-O-Laka (n = 28), Mark

Reef (n = 2) and Reef 42 (n = 2). Turtles were transported by boat to the research facilities provided by the University of Hawaii's Marine Fisheries Laboratory. Upon arrival, turtles were measured, tagged, and weighed following techniques previously described (Bjorndal and Balazs 1983). Dorsal and ventral barnacles (*Chelonibia testudinata*) were counted, and an evaluation based on the size, number, and location of the tumors was also made in turtles with GTFP. Turtles were coded by degree of tumor severity on a scale of 1-4, 4 being the most severe case (Balazs 1991).

Collection of Blood

Blood specimens (5-10 ml) were collected by venipuncture with a 12-ml syringe and a 18-g needle. Samples were taken from the bilateral dorsal cervical sinuses (Balazs 1985, Bennett 1986), placed in labeled test tubes, allowed to clot at 4°C, and centrifuged at 2,000 rpm for 10 minutes. The serum was separated from the clot by pipetting into two different labeled, screw-capped vials, one vial for arbovirus isolation and serology and another vial for future reference. Serum vials were stored in sealed, double plastic bags and frozen at -70°C in a Revco® ultrafreezer.

Virus Isolation

A set of nasopharyngeal and cloacal swabs were taken from all individuals and immediately placed in labeled vials containing a virus isolation diluent (VID) consisting of isotonic buffered salt solution, 0.1% nutrient broth, 0.003% gelatin, and 50 µg/ml gentamicin sulphate. The vials were frozen at -70°C prior to their submission to the Oregon State University Laboratory for Fish Disease Research, Newport, Oregon.

General Bacteriology

Two sets of nasopharyngeal and cloacal swabs were taken for bacterial isolation and identification. A set of swabs was immediately placed in labeled vials containing Cary-Blair Transport Medium; the second set was placed in Modified Stuart's Bacterial Transport Medium (Culturette,® Baxter Scientific Products, McGraw Park, IL 60085-6787). All specimens were express-mailed to the Department of Health Services Office of the County Veterinarian Laboratory in San Diego, California, for bacterial isolation and identification. This laboratory has extensive experience in working with bacteria isolated from marine mammals and reptiles.

Chlamydial Antigen

Cloacal swabs were collected from all 32 green turtles and immediately placed in labeled vials containing Bovarnick's Buffer Transport Medium with 10% fetal calf serum (Spencer and Johnson

1983). Sealed tubes were frozen at -70°C for the diagnosis of *Chlamydia* spp. antigen.

Hemoparasites

Thin smears from fresh blood were made, air dried, and immediately fixed in absolute methanol. Slides were numbered and stored for later staining and examination of hemoparasites.

Histopathology and Electron Microscopy

Two tissue biopsies from the prickle cell layer of the epidermis of early growths were collected from each of 10 green turtles manifesting fibropapillomas. One biopsy specimen was fixed in 10% neutral buffered formalin for histopathologic evaluation. A second biopsy was fixed in phosphate buffered 4.0% formaldehyde -1.0% glutaraldehyde and held at 4°C until processed for both light and transmission electron microscopy (McDowell 1978).

Laboratory Techniques

Arbovirus Isolation

Virus assays, titrations, and neutralizing antibody tests were conducted as described by McLean et al. (1985). For virus isolation, serum specimens were mixed with 0.9 ml of field diluent consisting of 20% heat-inactivated fetal bovine albumin-fraction-1 and antibiotics (GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY 14072). A sample of 100 μl of each dilution was inoculated into serially propagated VERO cell cultures® (C1008, African green monkey kidney, American Type Culture Collection, Rockville, MD 20852) grown in 25-cm² flasks, and allowed to absorb for 1 h in 5% CO_2 at 37 C. Cultures were overlaid with nutrient medium containing 1% Noble agar® (Difco Laboratories, Detroit, MI 48232) and 1:25,000 neutral red dilution and incubated at 37°C in 5% CO_2 for 7-10 days or until plaques could be counted. Flasks were examined every day for the identification of plaques.

Arbovirus Serology

After the virus isolation trials, serum samples were heat-inactivated at 56°C for 30 min and tested by the plaque-reduction neutralization (N) antibody test. Equal volumes of serum and challenging viruses (approximately 150 plaque-forming units per 100 μl) were mixed and then incubated overnight at 4°C . A sample of 100 μl from the mixture was added to monolayers of Vero cell cultures grown in 6-well plastic plates and absorbed at 37°C for 1 h in 5% CO_2 and treated as above. Samples presenting $\geq 80\%$ reduction or a linear neutralization index of $10^{0.8}$ log of plaque counts when compared with controls were considered positive.

Other Virus Isolations

Swabs transported in VID were defrosted and diluted each with 1 ml of Eagle's Minimum Essential Medium containing 200 IU penicillin and 200 μ g streptomycin/ml, 10% fetal bovine serum, and 200 mM HEPES buffer. Dilutions were vigorously mixed with a vortex mixer and inoculated onto four fish cell lines commonly used in fish virology. These cell lines were originated from *Oncorhynchus tshawytscha* (CHSE-214, ATCC CRL 1681), *O. mykiss* (RTG-2, ATCC CCL 55), *Pimephales promelas* (FHM, ATCC CCL 42), and *Cyprinus carpio* (EPC). A sample of 0.05 ml of each preparation was inoculated onto each of four wells of a 96-well plate of each cell line. These cultures were incubated at 18°C and observed. Culture fluid and cell debris from wells displaying cytopathic effects were diluted at 1:100 then transferred to fresh cell cultures and further incubated and observed. Final subcultures were incubated for 28 days.

Bacterial Isolations

Material from nasopharyngeal and cloacal swabs and tissue specimens were cultured on different selective and non-selective media including blood agar (5% sheep blood), MacConkey agar, trypticase soy agar, and seawater agar. To support organisms with an obligate salt requirement, all media contained 3% NaCl. Plates were incubated at 25°C aerobically, then examined after 24 h. If there was little or no growth, plates were reincubated for an additional 24 hr. Colonies demonstrating different characteristics (color, texture, growth patterns) were treated as separate organisms and isolated on blood agar plates for identification. Microorganisms were gram stained and separated based on gram reaction and morphology. Gram negative bacteria were biochemically identified in the laboratory. Also, the API 20E System® (Sherwood Medical, Plainview, New York 11803, USA) was used to identify microorganisms. Gram positive organisms were identified based on colonial and cellular morphology and staining characteristics (Balows and Hausler 1981).

For the identification of *Vibrio* spp., swabs were placed in 10 ml double-strength alkaline peptone broth and incubated overnight at 42°C. Then, 0.5 ml of the broth was transferred to 10 ml of 96S4YL medium, and incubated at 37°C for 24 h. The growth appearing in the incubated medium was plated on Thiosulfate Citrate Bile Salts Sucrose agar. Plates were incubated at 37°C for 24 hr and examined for growth of *Vibrio* spp. Typical colonies were biochemically identified or tested by using the API 20E system (Ogg et al. 1989).

Chlamydial Antigen

A direct-binding monoclonal based enzyme immunoassay, the KODAK SURECELL™ Chlamydia Test Kit (Eastman Kodak Company,

Clinical Products Division, Rochester, NY 14650), was used for the rapid detection of chlamydial antigen. The test was performed according to the manufacturer's directions, excepting for centrifugation of the transport media containing fecal material.

Hemoparasites

Blood smears were stained using Diff-Quik® (American Scientific Products, Inc., McGraw Park, IL 60085). Stained slides were examined for *Trypanosoma* spp., intraleukocytic *Leucocytozoon*-like hemoparasites and microfilariae under low power (100x) for 10 min each. *Haemoproteus* spp., *Hepatozoon* spp., *Plasmodium* spp., and hemogregarines were examined at high power, oil immersion (1000x and 5000x) light microscope during 10-15 min or 50 fields (10,000 erythrocytes).

Histopathology and Electron Microscopy

For histopathology, fixed fibropapilloma lesions were embedded in paraffin, sectioned 6- μ m thick, and stained with haematoxylin and eosin. The skin lesions for electron microscopy were washed with 0.2 M Sorenson's phosphate buffer pH 7.3 and were postfixed in 1.0% osmium tetroxide for 1 h. The tissues were washed through two changes of ddH₂O, dehydrated through a graded acetone series, infiltrated with and embedded in Medcast-Araldite 502 Resin® (Ted Pella Inc., Redding, CA). Semithin, 1-2 μ m survey sections were cut from the blocks, stained with Methylene Blue-Azure II-Basic Fuchsin, and examined with a light microscope (Hayat 1986). Ultrathin sections from the tumor biopsies of two turtles were placed on copper grids, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope by Dr. Robert E. Lee (Colorado State University, Department of Anatomy and Neurobiology, Fort Collins, CO 80523).

RESULTS

Green Turtles

Ten green turtles (31%), with an average weight of 19 ± 2.0 kg (range 11.8-22.3 kg) and a mean straight carapace length of 55.3 ± 2.2 cm (range 44.5-68.3 cm), presented multiple cutaneous and conjunctival fibropapillomas. The degree of tumor severity in the turtles trapped varied from mild to very severe (Figure 1). The incidence of tumors in green turtles captured and tagged in Ahu-O-Laka was 29% (8/20). Two turtles trapped at Reef 42 had tumors, and two trapped at Mark Reef were free of GTFP. Twenty two (69%) green turtles free of GTFP, with an average weight of 12.3 ± 1.0 kg (range 7.7-25.5 kg) and a mean straight carapace length of 44.0 ± 1.1 cm (range 37.4-55.2 cm), were also trapped.

During this study, barnacles were found in the carapace and plastron of 15/22 (68%) green turtles free of GTFP and on 9/10 (90%) turtles afflicted with GTFP. The dorsal barnacle average count in the green turtles afflicted by GTFP was 8.2 ± 13.5 (range 0-59) and ventral barnacle count averaged 16.3 ± 21.7 (range 0-75). The group free of the disease had an average count of dorsal barnacles of 12.8 ± 12.7 (range 0-41) and ventral barnacles of 21.8 ± 18.2 (range 0-65).

Arbovirus Isolation and Serology

None of the 32 serum specimens yielded arboviruses after flasks were examined daily for 10 days for the identification of plaques.

Other Virus Isolations

A total of five tumor biopsies, and 30 nasopharyngeal and cloacal swabs were inoculated in fish cell lines. None of the culture fluids and cell debris produced cytopathic effects after the 28-day incubation period. All early cell death in several specimens was caused by toxic materials in the original inoculum.

Bacterial Isolations

The bacterial cultures of nasopharyngeal and cloacal swabs taken from sea turtles yielded 28 gram negative bacteria, 5 gram positive cocci, *Bacillus* spp., and diphtheroids. *Pseudomonas fluorescens* was most frequently isolated (22/32). This was followed by *P. putrefaciens* (21/32), *Vibrio alginolyticus* (16/32), non-hemolytic *Streptococcus* (16/32), *V. damsela* (15/32), and *V. fluvialis* (15/32). Other common isolates included *Micrococcus* spp., *Acinetobacter anitratus*, *Citrobacter freundii*, and *P. maltophila* (Table 1).

Potentially pathogenic bacteria for sea turtles isolated in this study included *Citrobacter* spp., *Enterobacter* spp., *P. aeruginosa*, *P. maltophila*, *Staphylococcus epidermidis*, and *Vibrio alginolyticus*. The microorganisms isolated only from sea turtles with GTFP were *Hafnia alvei*, *Klebsiella oxytoca*, and *Pseudomonas stutzeri*. These isolates came from three different individuals. Alpha-streptococci, *Escherichia coli*, and *Citrobacter diversus* were isolated from a single turtle free of GTFP. During the field studies, an apparently healthy turtle presented sloughing of the skin in the front flippers with no other apparent lesions. *Staphylococcus epidermidis*, *Vibrio damsela*, and *V. fluvialis* were isolated from swabs taken underneath the epidermis.

Chlamydial Antigen

From 32 sea turtle cloacal swabs examined, four were chlamydia antigen positive. No attempts were made to culture and isolate the microorganism.

Hemoparasites

No hemoprotozoans, microfilaria, or hemogregarins were identified under low or high power light microscopy.

Histopathology and Electron Microscopy

Tissue samples from 10 green sea turtles were examined for histopathology. The primary lesions in these animals were characterized by a hyperplasia of squamous epithelial cells and mesodermal proliferation with a marked degree of orthokeratotic hyperkeratosis. In many places the epithelial cells formed outward papillary projections; in one turtle, however, the squamous epithelial cells did appear to have more of a downward growth. There was an extensive proliferation of fibroplastic cells underneath the squamous hyperplastic process. Necrotic foci were observed in the squamous epithelial cells of the epidermis in 3/10 sea turtle tumors. In many areas the lesions were flattened or plaque-like similar to a ballooning degeneration, whereas in others there was a papillary configuration with intercellular bridges in the epithelial cells. In some areas, lesions progressed from epidermis to dermis. Epithelial cells, especially in the outer edges of the epidermis, were markedly hypertrophic, presenting large basophilic intranuclear bodies and extensive intracytoplasmic vacuolization.

In 8 of 10 cases, granulomas containing a yellow nonstaining cuticle-like material both within normal dermis and within hyperplastic mesodermal areas were observed. These structures were identified as spirorchid trematode eggs. One of the sections contained a specimen of the piscicolid leech *Ozobranchus branchiatus* attached to the surface of the tumor. At least 9/10 of the specimens contained organisms compatible with yeast or algal growth and two contained fungi. These yeast/algae and fungi were present on the surface and between layers of keratin. Seven of the specimens presented bacterial organisms on the surface of the keratinized layer and 8/10 contained mites on the surface of the tumors (Figure 2; Table 2).

Light microscopy examination of eight tissues stained with Methylene Blue-Azure II-Basic Fuchsin revealed the same lesions described for the histopathologic examination. No further electron microscopic analysis was made.

The electron microscopic findings of two turtle specimens included multiple epidermal folds associated with dermal proliferation and epidermis thicker than normal. Dermal papillae were projected toward the epidermis, and the intercellular spaces were enlarged. Increased numbers of subcellular organelles, endoplasmic reticulum, and mitochondria, hypertrophy and hyperplasia of the stratum spinosum with pleomorphic cells were also identified. In both cases, granules with electron-dense bodies about 150 nm in diameter similar to viral particles were

observed in the more superficial cells of the epidermis. Ultrastructurally, in deeper areas of the dermis cellular clusters were identified around the blood vessels including lymphocytes, heterophils, plasma cells, and macrophages. Trematode eggs with golden yellow capsules were observed in one specimen within the dermal vessel surrounded by multinucleated giant cells.

DISCUSSION

Barnacles are one of the most frequently encountered epizoic associates on the carapace, plastron, and head of sea turtles and have little if any detrimental effect except in cases where incrustations on the head interfere with the action of eyelids (Lauckner 1985). These epizoics have not been associated with lesions or other signs of pathology in green turtles. Although their incidence was higher in turtles affected by GTFP (90%) than in turtles free of the disease (68%), their role in the metaplasia process is unknown.

Virus Isolations

Although virus isolations attempted in fish cell cultures and Vero-cell cultures were negative, the search for a virus should continue. Papovaviruses are the infectious agents causing fibropapillomas in other species. These viruses become incorporated in the cell genome or DNA. The viral particles are extremely difficult to observe by light or electron microscopy and have not been isolated in tissue culture.

Bacterial Isolations

The normal bacterial flora of green turtles and their role and persistence have not been characterized. This study provided a beginning data base on bacterial isolates in free-ranging, juvenile green sea turtles afflicted with GTFP and those free of the disease. A wide range of bacteria was isolated from both groups. Many of these organisms are considered normal flora in terrestrial reptiles, others are known to be potentially pathogenic to green turtles, and several are considered saprophytic. *Acinetobacter* spp., *Citrobacter* spp., *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Vibrio alginolyticus* were consistently isolated from both sea turtle groups. These and other bacteria are known to act as opportunistic pathogens invading tissues damaged by trauma. These bacteria have been associated in farmed sea turtles with ulcerative shell disease, traumatic ulcerative dermatitis, necrotic dermatitis, ulcerative stomatitis, obstructive rhinitis, keratoconjunctivitis, septicemia, bronchopneumonia, and osteomyelitis (Lauckner 1985, Glazebrook and Campbell 1990a).

Citrobacter freundii is known to cause septicemic cutaneous ulcerative disease in other turtle species (Cooper 1981, Lauckner 1985). This microorganism was isolated in 9/32 sea turtles sampled during the present survey. The role of other bacteria, including coliforms and *Enterobacter* spp., is unknown although they have been incriminated in local and generalized disease in terrestrial reptiles (Cooper 1981, Hoff et al. 1984, Lauckner 1985, Glazebrook and Campbell 1990a).

Klebsiella oxytoca, *Pseudomonas stutzeri*, and *Hafnia alvei* were isolated from turtles afflicted with GTFP. These bacterial species are distributed in aquatic environments and are commonly isolated from the oral cavity of terrestrial reptiles as saprophytes or commensals. *Hafnia alvei* has been isolated from animal clinical specimens and the environment. Their pathogenic significance is unknown (Carter and Chengappa 1991). Further studies are necessary to determine the role of these and other bacteria in the epizootiology of GTFP.

Bacteria may act as secondary invaders of skin lesions produced by a herpesvirus. Gram positive cocci and gram negative rods were observed in the upper portion of the keratin layer in skin areas of green turtles affected with grey-patch disease (Lauckner 1985). During this study, histological examination of 3/11 turtle specimens revealed epithelial necrosis associated with the presence of bacteria and fungi in the keratin layer. Possibly, secondary infections may induce ulcerative lesions of tumors leading to a more severe condition in these animals.

Saprophytic bacteria including *Bacillus* spp., *Micrococcus* spp., and *Proteus* (*Providencia*) spp. are considered part of the normal skin flora of sea turtles (Glazebrook and Campbell 1990a).

Chlamydial Antigen

Chlamydial infections in reptiles were unknown until recently. The first isolations of *Chlamydia* spp. were reported in 1988 from a chameleon (*Chamaeleo fischeri*) and puff adders (*Bitis arietans*) (Frye 1991). Further research and isolation attempts are required before definite conclusions can be drawn related to the possibility of chlamydial infections in Hawaiian *Chelonia mydas*.

Histopathology and Electron Microscopy

The histopathologic lesions identified in these samples have been previously described by other investigators (Jacobson et al. 1989, Balazs and Pooley 1991). The intranuclear bodies observed in the epithelial cells of several tumors appear to be large nucleoli; however, these are compatible with intranuclear basophilic inclusion bodies described for the papilloma virus. The fibropapilloma lesions in these sea turtles are compatible

with the viral infection described for cervids. Apparently, the etiologic agent is a specific papilloma virus of the family Papovaviridae. The viral particles, however, could not be observed under light microscopy.

The electron microscopic findings of two turtle specimens were similar to the molecular examination of fibropapillomas reported by Jacobson et al. (1989). The most relevant finding in the specimens examined was the appearance of virus-like structures. These structures are found in both normal and diseased skin from green turtles and, although they have superficial characteristics of a herpesvirus, these are part of the normal keratinization process of the skin. Herpesvirus intranuclear inclusions have been demonstrated in green turtles from Florida (Jacobson et al. 1991). Mites (Acarina) (Glazebrook and Campbell 1990a) and unidentified black "mites" (Balazs 1985) have been reported embedded in the skin of green sea turtles. All tumor biopsies collected contained mites on the surface. The association of these ectoparasites to GTFP is unknown and needs further research.

The parasites identified in these turtles have been previously reported in green and other species of sea turtles. Most of these reports have concentrated on taxonomic description with little or no information on infestation levels and associated clinical and pathologic signs (Lauckner 1985, Glazebrook and Campbell 1990b). Although foreign organic materials (leeches, barnacles, algae) present on the skin presumably are not associated with the etiology of GTFP in Hawaiian green turtles, these have been suggested as possible etiologic agents in other turtle populations (Balazs 1980).

RECOMMENDATIONS

The environmental conditions and the relationship of GTFP to contaminants/toxic substances in known green turtle habitats should be investigated. Specifically, the analysis of contaminants including pesticides, heavy metals, and other toxicants is recommended.

Thorough necropsies of freshly dead animals should be performed, and tissues should be collected for histopathology, bacteriology, virology, parasitology, and toxicology. These pathologic studies could identify peripheral diseases. Chronic effects of stress can be also determined by adrenal structure in necropsied turtles. These studies may be complemented by measuring blood cortisol in trapped animals afflicted and free of GTFP.

A study describing taxonomy, biology, and life cycles of epizoic algae, barnacles, and ectoparasites and their role in GTFP should be initiated. For example, mites reported in Australian green turtles and black "mites" reported in Hawaiian

green turtles were mentioned briefly in the literature, but it is unknown if the authors refer to the same ectoparasite. A taxonomic description of this and other ectoparasites is necessary.

Epidemiologic and statistical analyses of data collected in previous years from Hawaiian green turtles related to GTFP should be performed to provide a basic research framework. These studies can describe temporal trends and other epidemiologic features giving a better understanding on the natural history and etiology of GTFP.

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Table 1.--Bacteria isolated from 32 green sea turtles (*Chelonia mydas*) afflicted or free of fibropapillomas (GTFP) using two different transport media, Kanehoe Bay, Island of Oahu, Hawaii, 1991.

Bacteria	Turtles			
	No. w/GTFP	No. w/o GTFP	Total	(%)
<i>Acinetobacter anitratus</i>	2	8	10	(31)
<i>A. lwoffii</i> ^a	1	1	2	(6)
<i>Bacillus</i> spp.	2	3	5	(16)
<i>Citrobacter diversus</i> ^{a,c}	0	1	1	(3)
<i>C. freundii</i> ^a	5	4	9	(28)
Diphtheroids	2	6	8	(25)
<i>Enterobacter aerogenes</i> ^a	1	2	3	(9)
<i>E. agglomerans</i>	0	2	2	(6)
<i>E. cloacae</i>	1	1	2	(6)
<i>Escherichia coli</i> ^{a,d}	0	1	1	(3)
<i>Hafnia alvei</i> ^{a,d}	1	0	1	(3)
<i>Klebsiella pneumoniae</i> ^b	0	2	2	(6)
<i>K. oxytoca</i> ^a	1	0	1	(6)
<i>Lactobacillus</i> spp.	1	2	3	(9)
<i>Micrococcus</i> spp.	3	7	10	(31)
<i>Morganella morganii</i> ^a	0	1	1	(3)
<i>Proteus mirabilis</i> ^a	1	2	3	(9)
<i>P. penneri</i> ^a	0	2	2	(6)
<i>P. vulgaris</i> ^{a,c}	0	1	1	(3)
<i>Providencia alcalifaciens</i> ^c	0	2	2	(6)
<i>Pseudomonas aeruginosa</i>	1	4	5	(16)
<i>P. fluorescens</i>	7	15	22	(69)
<i>P. maltophilia</i>	5	5	10	(31)
<i>P. putida</i>	2	4	6	(19)
<i>P. putrefaciens</i>	5	16	21	(66)
<i>P. stutzeri</i> ^d	1	0	1	(3)
<i>P. visicularis</i> ^{b,d}	0	1	1	(3)
<i>Staphylococcus aureus</i> ^b	0	2	2	(6)
<i>S. epidermidis</i> ^b	1	6	7	(22)
<i>Streptococcus alpha</i> ^d	0	1	1	(3)
<i>S. non-hemolytic</i>	3	13	16	(50)
<i>Vibrio alginolyticus</i>	4	12	16	(50)
<i>V. damsela</i>	4	11	15	(47)
<i>V. fluvialis</i>	6	9	15	(47)
<i>V. spp.</i> ^{b,c}	0	1	1	(3)

^aIsolated from cloacal swabs but not nasopharyngeal swabs.

^bIsolated from nasopharyngeal swabs but not cloacal swabs.

^cIsolated from Stuart's transport medium but not Cary-Blair.

^dIsolated from Cary-Blair transport medium but not Stuart's.

Table 2.--Summary of organisms identified by light microscopy on the surface of skin tumors of Hawaiian green sea turtles (*Chelonia mydas*), Kaneohe Bay, Island of Oahu, Hawaii, 1991.

Turtle No.	Bacteria	Algae/ yeast	Fungus	Mites	Adult leech	Trematode eggs-dermis	Epithelial necrosis
003	+	+		+		+	+
008		+		+	+	+	
009	+	+		+		+	+
016	+		+			+	
024	+	+		+		+	
028	+	+	+	+		+	
029		+		+			
030	+	+		+		+	+
031		+					
032	+	+					

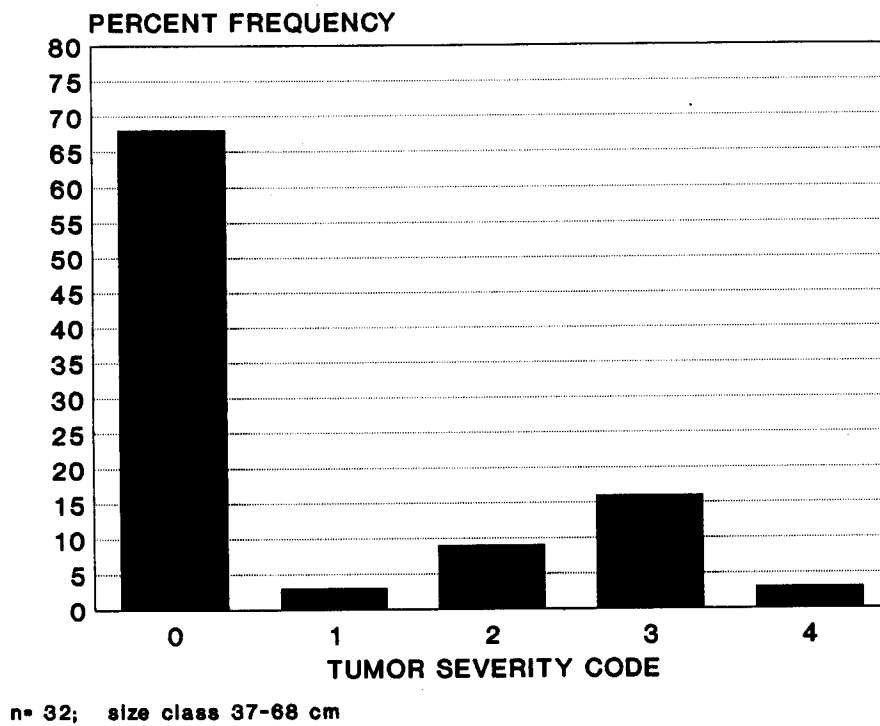


Figure 1.--Tumor severity and frequency in green turtles (*Chelonia mydas*) trapped at Kaneohe Bay, Island of Oahu, Hawaii, 1991 (code 0 = an absence of tumors).

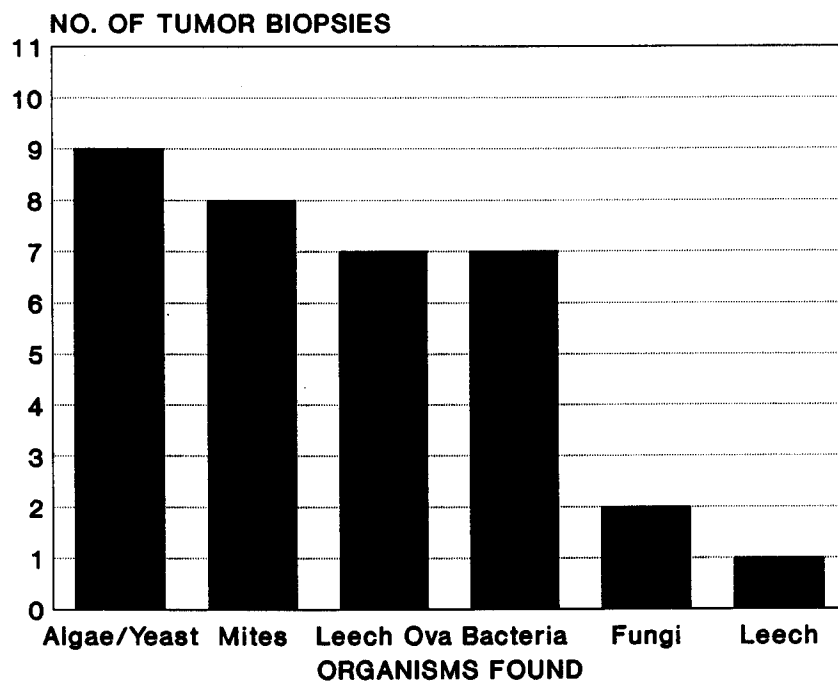


Figure 2.--Organisms identified in tumor biopsies collected from Hawaiian green sea turtles (*Chelonia mydas*), Kaneohe Bay, Island of Oahu, Hawaii, 1991.